

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)

THIS PAGE BLANK (USPTO)



Practitioner's Docket No. U012104-2

PATENT

1653#18
3/13/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: RAMA MUKHERJEE, ET AL.

Serial No.: 09/248,382

Group No.: 1653

Filed: FEBRUARY 10, 1999

Examiner: F. MOEZIE

For: NOVEL PEPTIDE ANALOGS FOR THE TREATMENT OF CANCER

Assistant Commissioner for Patents

Washington, D.C. 20231

TRANSMITTAL OF CERTIFIED COPY

Attached please find the certified copy of the foreign application from which priority is claimed for this case:

Country: INDIA

Application
Number: 343/DEL/98

Filing Date: 11 FEBRUARY 1998

RECEIVED
FEB 21 2002
TECH CENTER 1600/2900

WARNING: "When a document that is required by statute to be certified must be filed, a copy, including a photocopy or facsimile transmission of the certification is not acceptable." 37 C.F.R. 1.4(f) (emphasis added).

CERTIFICATE OF MAILING (37 C.F.R. 1.8a)

I hereby certify that this correspondence is, on the date shown below, being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Date: February 4, 2002

Signature

Janet I. Cord

(type or print name of person certifying)

(Transmittal of Certified Copy—page 1 of 2) 5-4

THIS PAGE BLANK (USPTO)


SIGNATURE OF PRACTITIONER

Reg. No. 33,778

Janet I. Cord

(type or print name of practitioner)

Tel. No.: (212) 708-1935

P.O. Address

Customer No.: 00140

c/o Ladas & Parry
26 West 61st Street
New York, N.Y. 10023

NOTE: "The claim to priority need be in no special form and may be made by the attorney or agent, if the foreign application is referred to in the oath or declaration, as required by § 1.63." 37 C.F.R. 1.55(a).

THIS PAGE BLANK (USPTO)



**GOVERNMENT OF INDIA
MINISTRY OF COMMERCE & INDUSTRY,
PATENT OFFICE, DELHI BRANCH,
W-5, WEST PATEL NAGAR,
NEW DELHI-110 008.**

*I the undersigned being an officer duly authorised in
accordance with the provision of the Patent Act, 1970 hereby certify
that annexed hereto is the true copy of the Application and
Complete Specification filed in connection with Application for
Patent No.343/Del/98 dated 11th February 1998.*

RECEIVED
FEB 21 2002
TECH CENTER 1600/2900

Witness my hand this 26th day of September 2001.


(H.C. BAKSHI)

Deputy Controller of Patents & Designs.

THIS PAGE BLANK (USPTO)

FORM 1A
THE PATENTS ACT, 1970
APPLICATION FOR PATENT

0343-98

11 FEB 1998

By the assignee or Legal Representative of the True and First Inventor.
[See Section 7]

We, NATIONAL INSTITUTE OF IMMUNOLOGY, a Society registered under the Indian Society's Registration Act XXI, of Aruna Asaf Ali Marg, New Delhi-110 067, India and Dabur Research Foundation, of 22, Site IV Sahibabad, Ghaziabad-201 010. (U.P).

hereby declare:-

i) that we are in possession of an invention for
"NOVEL PEPTIDE ANALOGS FOR THE TREATMENT OF CANCER"

ii) that we claim to be the assignees of RAMA MUKHERJEE, an Indian citizen of National Institute of Immunology, of Aruna Asaf Ali Marg, New Delhi-110 067 and MANU JAGGI, SUDHANAND PRASAD AND ANAND C. BURMAN, all Indian citizens, of Dabur Research Foundation, 22, Site IV Sahibabad, Ghaziabad-201 010. (U.P).

who claim and are believed to be the true and first inventors thereof;

iii) that the complete specification filed with this application and any amended specification which may hereafter be filed in this behalf will be true of the invention to which this application relates;

iv) that we believe that we are entitled to a patent for the said invention having regard to the provisions of The Patents Act, 1970;

v) that to the best of our knowledge, information and belief, the facts and matters stated herein are correct and that there is no lawful ground of objection to the grant of patent to us on this application.

We, request that a patent may be granted to us for the said invention.

We request that all notices, requisitions, and communications relating to this application may be sent to REMFRY & SAGAR, Attorneys-at-Law, Remfry House, 8, Nangal Raya Business Centre, New Delhi-110 046.

Dated this 11th day of February, 1998

OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANTS

TO
THE CONTROLLER OF PATENTS
THE PATENT OFFICE
DELHI

DUPLICATE

THIS PAGE BLANK (USPTO)

343/22/98

THE PATENTS ACT 1970

COMPETE SPECIFICATION

(Section 10)

11 FEB 1999

Govt. of India Patent Office
New Delhi
Received Rs. 200/- in cash
Chaitanyam/M.O.A.P.O./D.D.
17 FEB 1999
File No. 928
Examination Fee
2

NOVEL PEPTIDE ANALOGS FOR THE TREATMENT OF CANCER

National Institute of Immunology and Dabur Research Foundation, a Society registered under the Indian Society's Registration Act XXI, of Aruna Asaf Ali Marg, New Delhi - 110067, India and of 22 Site IV Shaibabad, Gaziabad - 2001 010, UP, India, *respectively*.

The following specification particularly describes and ascertains the nature of this invention and the manner in which it is to be performed:-

NOVEL PEPTIDE ANALOGS FOR THE TREATMENT OF CANCER

FIELD OF INVENTION

This invention relates to design, synthesis and anticancer activity of novel peptide analogs of vasoactive intestinal peptide, somatostatin, bombesin and substance P. Neuropeptide analogs are increasingly implicated in treatment of cancers.

BACKGROUND OF THE INVENTION

The neuropeptides vasoactive intestinal peptide and bombesin exert physiological effects by binding to specific receptors present on cells in the gastrointestinal tract and central nervous system. Bombesin is an amphibian peptide that has a structure closely related to that of several mammalian peptides, including Gastrin Releasing Peptide (GRP) and Neuromedins I and C. It was discovered in 1970 as a potent smooth muscle contracting agent of nonmammalian origin first isolated from amphibians in (Erspamer et al., J Pharm. Pharmacol. 22:275 (1970)). Bombesin, GRP and related peptides exert their in vivo effects by binding to specific receptors present on cells of the gastrointestinal tract, the central nervous system and tumors. The invention relates to novel bombesin analogs which act as antagonist of bombesin or related peptides such as gastrin releasing peptide. By blocking the binding of bombesin-like peptides to their receptors, these antagonists block the physiological effects of these peptides and inhibit the growth of tumor cells that respond to growth-promoting action of bombesin. Thus these antagonists have therapeutic use in the treatment or prevention of cancer and in controlling physiological effects in gastrointestinal disorders and in modulating responses of the central nervous system.

The invention also relates to novel vasoactive intestinal peptide analogs which act as antagonists of VIP by blocking the binding of VIP to its cognate receptors. By blocking the binding of VIP to its receptor these analogs inhibit the growth of tumor cells that respond to the

growth-promoting action of VIP. VIP is a 28-amino-acid neuropeptide, which has been implicated as a major growth promoting factor during embryonic growth. In cancer cells, previous studies have implied that VIP can serve as an autocrine growth factor in lung tumors (Gozes et al. Biomed Res. 13 (Suppl. 2), 37. (1992)).

In our US Application 08/727,679 we have described the role of neuropeptides in cancer. High affinity and moderate affinity receptors for vasoactive intestinal peptide and somatostatin, high affinity receptors for bombesin and moderate affinity receptors for substance P were demonstrated on human colon adenocarcinoma cells. It was further demonstrated that peptide analogs to the above neuropeptides could actively and selectively induce cell death in the cancer cells. A formulation of peptide combination termed MuJ-7 has also been described which causes tumor regression in xenotransplanted nude mice. The individual constituent peptides of MuJ-7 were demonstrated to have anticancer activity.

Our further research work has led to the designing and testing of novel structural analogs of VIP receptor binding inhibitor and bombesin antagonist (both constituent peptides of MuJ-7 formulation) which have been designed to render conformational constraints and higher stability to the peptides while maintaining their anticancer activity. Substitutions and / or eliminations have been incorporated into VIP₂ and BOM₁ sequences keeping in mind not to alter amino acids known to offer conformational constraint and stability to the peptide. In order to introduce conformational constraints, unusual amino acids such as cyclic and acyclic dialkylated Glycines have been incorporated into the peptide backbone. Examples of such amino acids are Aib, Me, Leu, Di-ethylglycine and its higher homologs, 1-amino cycloalkane carboxylic acids.

SUMMARY OF THE INVENTION

The invention provides novel peptides for the treatment of cancer. The VIP receptor binding inhibitor (Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys) has been shown in our previous studies to be a selective cytotoxic peptide for cancer cells having receptors for vasoactive intestinal peptide. In our present studies we have designed new analogs of this sequence in order to introduce conformational constraints and resist enzymatic degradation. The peptides thus synthesised were

DT-11	Aib-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH
DT-12	D-Leu-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH
DT-13	Leu-Met-Tyr-Pro-Thr-D-Tyr-Leu-Lys-OH
DT-14	Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH
DT-15	Leu-Met-D-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH
DT-16	D-Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH
DT-18	Aib-Met-Tyr-Pro-Thr-Tyr-Dxg-Lys-OH
DT-19	D-Leu-Met-Tyr-Pro-Thr-Tyr-Dxg-Lys-OH

where Dxg represents Cyclic and acyclic dialkylated Glycines e.g., Di-ethylglycine and its higher homologs, 1-amino cycloalkane carboxylic acids and Aib represents alpha-Amino isobutyric acid.

The bombesin antagonist (D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH₂) has been shown in our previous studies to be a selective cytotoxic peptide for cancer cells having receptors for bombesin. In our present studies we have designed new analogs of this sequence in order to introduce conformational constraints and resist enzymatic degradation. The peptides thus synthesised are

DT21	D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH ₂
------	---

DT22	D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH ₂
DT-23	D-Phe-Gln-Trp-Aib-Val-Gly-His-Leu-NH ₂
DT-24	D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH ₂
DT-25	D-Phe-Gln-Trp-Ala-Val-Gly-His-Ile-NH ₂
DT-26	D-Phe-Gln-Trp-Aib-Val-Gly-His-Ile-NH ₂
DT-27	D-Phe-Gln-Trp-Ala-Val-Aib-His-Ile-NH ₂

where Aib represents alpha-Amino isobutyric acid.

The Substance P analog (D-Arg-Pro-Lys-Pro-Ala-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH₂) has been shown in our previous studies to be a selective cytotoxic peptide for cancer cells having receptors for Substance P. In our present studies we have designed new analogs of this sequence in order to introduce conformational constraints and resist enzymatic degradation. The peptides thus synthesised are

DT-31	Aib-Met-Gln-Trp-Phe-Aib-NH ₂
DT-32	Dxg-Met-Gln-Trp-Phe-Aib-NH ₂
DT-33	D-Leu-Met-Gln-Trp-Phe-Aib-NH ₂
DT-34	D-Arg-Pro-Lys-Pro-Aib-Gln-D-Trp-Phe-D-Trp-Aib-Leu-NH ₂
DT-35	Arg-Pro-Aib-Pro-D-Phe-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH ₂

where Dxg represents Cyclic and acyclic dialkylated Glycines e.g., Diethylglycine and its higher homologs, 1-amino cycloalkane carboxylic acids and Aib represents alpha-Amino isobutyric acid.

The Somatostatin analog Ala-Gly-Cys-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys (disulfide bridges: 3-14) has been shown in our previous studies to be a selective cytotoxic peptide for cancer cells having receptors for Somatostatin. In our present studies we have

designed new analogs of this sequence in order to introduce conformational constraints and resist enzymatic degradation. The peptides thus synthesised are

DT-61 Ala-Aib-Cys-Lys-Asn-Phe-Phe-D-Trp-lys-Thr-Phe-
 Thr-D-Ser-Cys (3-14 Disulphide bond)

where Aib represents alpha-Amino isobutyric acid.

The Somatostatin analog (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂) has been shown in our previous studies to be a selective cytotoxic peptide for cancer cells having receptors for Somatostatin. In our present studies we have designed new analogs of this sequence in order to introduce conformational constraints and resist enzymatic degradation. The peptide thus synthesised is

DT-71 D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Aib-Thr-NH₂

where Aib represents alpha-Amino isobutyric acid.

EXAMPLE 1

The cytotoxic activity of the peptides synthesised was tested on eight human tumor cell lines namely HT-29, SW620, PTC (all colon), PA-1 (ovary), A549 (lung), HBL100 (breast), MOLT-4 (leukemia) and DU145 (prostate). The tumor cells were collected at exponential growth phase and resuspended in medium (1.5×10^6 cells/ml in RPMI 1640 containing 10% FBS). 150µl of medium was added to the wells of a 96-well tissue culture plate (Nunc, Denmark) followed by 30µl of cell suspension. The plate was left in incubator (37°C, 5% CO₂) overnight. 20µl of the peptide (10^{-7} to 10^{-10} M concentration) was added to marked wells of the 96-well plate. Each concentration was plated in triplicates. 20µl of medium alone was added to control wells while wells without cells served as blanks. A total volume of 200µl was ensured in each well and plate was left in incubator (37°C, 5% CO₂). After 72 hours of

incubation an MTT assay was performed and percentage cytotoxicity was calculated with respect to control cells. The following Tables I to V show the results obtained.

TABLE - I

**Percent cytotoxicity of DT-1 analogs on
various tumor cell lines**

Cell line	DT11	DT12	DT13	DT14	DT15	DT16	DT18	DT19
<i>PA-1</i>	32	26	23	14	25	22	31	34
<i>PTC</i>	16	24	20	0	23	18	14	10
<i>MOLT-4</i>	30	42	39	30	33	31	36	23
<i>HBL100</i>	24	19	27	19	18	2	24	27
<i>A549</i>	29	36	32	42	37	33	27	25
<i>SW620</i>	13	0	18	12	28	22	26	33
<i>HT29</i>	0	7	6	6	11	20	38	30
<i>DU145</i>	7	19	1	5	10	0	18	24

TABLE - II

**Percent cytotoxicity of DT-2 analogs on
various tumor cell lines**

Cell line	DT21	DT22	DT23	DT24	DT25	DT26	DT27
<i>PA-1</i>	23	24	16	33	21	24	26
<i>PTC</i>	18	12	15	23	9	25	12
<i>MOLT-4</i>	25	17	29	8	23	20	13
<i>HBL100</i>	27	14	33	32	25	6	16
<i>A549</i>	16	22	23	30	25	13	18
<i>SW620</i>	25	33	34	38	31	38	32
<i>HT29</i>	24	35	43	44	40	27	28
<i>DU145</i>	10	22	25	32	33	24	0

TABLE III

Percent cytotoxicity of DT-3 analogs
on various tumor cell lines

Cell line	DT31	DT32	DT33	DT34	DT35
PA-1	24	ND	ND	ND	ND
<i>PTC</i>	21	32	23	16	18
<i>MOLT-4</i>	30	32	37	26	20
<i>HBL100</i>	15	16	15	21	15
<i>A549</i>	23	19	21	24	20
<i>SW620</i>	14	ND	ND	ND	ND
<i>HT29</i>	30	13	18	9	26
<i>DU145</i>	25	ND	ND	ND	ND

TABLE IV

Percent cytotoxicity of DT-6 analogs
on various tumor cell lines

Cell line	DT61
PA-1	45
<i>PTC</i>	22
<i>MOLT-4</i>	34
<i>HBL100</i>	26
<i>A549</i>	28
<i>SW620</i>	26
<i>HT29</i>	35
<i>DU145</i>	29

TAVLE V

**Percent cytotoxicity of DT-7 analogs
on various tumor cell lines**

Cell line	DT71
PA-1	ND
<i>PTC</i>	19
<i>MOLT-4</i>	37
<i>HBL100</i>	23
<i>A549</i>	18
<i>SW620</i>	ND
<i>HT29</i>	19
<i>DU145</i>	ND

EXAMPLE 2

A 0.5 mL of 2000 ppm of the VIP_{2a} was mixed with 1.0 ml freshly prepared liver homogenate to obtain a concentration of 1000 ppm. Sample preparations was incubated at 37° C and after time intervals of 0, 2, 5, 10, 20 and 30 minutes, 200µl of the preparation was aliquot and precipitated with equal volumes of acetonitrile. Incase of BOM₁ analogs, the sample preparations with a final concentration of 1000 ppm were incubated at 37° C and after time intervals of 0, 15, 30, 60, 90, 120 and 150 minutes, 200µl of the preparation was aliquot and precipitated with equal volumes of acetonitrile. The precipitate was pelleted by centrifugation at 10,000 g for 5 min and supernatant analyzed by HPLC. The percentage increase in the half-life of DT1 and DT2 analogs with reference to DT1 and DT2, respectively, as estimated by the mouse liver homogenate study, is shown in Tables VI and VII respectively.

Preparation Of Mouse Liver Homogenate

- 1 Healthy Balb/c mouse was sacrificed and dissected to expose liver.
- 2 The pulmonary artery was severed to drain blood and cold saline was perfused through portal vein till liver becomes pale white.
- 3 The liver was excised ,minced and passed through 60# sieve.
- 4 1.15% w/v KCl -0.01M phosphate buffer (pH 7.4) was added to make 20% w/v homogenate that was centrifuged at 4500 g for 15 min.
- 5 The supernatant was recovered and further centrifuged at 10,000 g to clarify the homogenate.

TABLE VI

Half life of DT-1 analogs with reference to DT-1 as determined by the mouse liver homogenate study

Peptide	Half-life (minutes)
DT-1	4.9
DT-11	15.4
DT-12	18.1

TABLE VII

Half life of DT-2 analogs with reference to DT-2 as determined by the mouse liver homogenate study

Peptide	Half-life (minutes)
DT-2	12.6
DT-22	15.06
DT-23	57.7
DT-24	38.5
DT-26	114.5
DT-27	292.5

WE CLAIM:-

1. A Peptide having the general formula $\text{Leu}^1\text{-Met}^2\text{-Tyr}^3\text{-Pro}^4\text{-Thr}^5\text{-Tyr}^6\text{-Leu}^7\text{-Lys}^8$.
2. A peptide according to claim 1, wherein any one or more of the amino acids at positions 1 to 8 is replaced by D_{xg} wherein D_{xg} represents cyclic and acyclic dialkylated Glycines such as Aib, Di-ethylglycine and its higher homologs and 1-amino cycloalkane carboxylic acids.
3. A peptide according to claim 1 or 2, wherein the peptide is used for the treatment of cancer of colon, breast, ovary, lung, prostate or leukemias.
4. A peptide having the general formula $\text{D-Phe}^1\text{-Gln}^2\text{-Trp}^3\text{-Ala}^4\text{-Val}^5\text{-Gly}^6\text{-His}^7\text{-Leu}^8\text{-NH}_2$.
5. A peptide according to claim 4, wherein any one or more of the amino acids at positions 1 to 8 is replaced by D_{xg} wherein D_{xg} represents cyclic and acyclic dialkylated Glycines such as Aib, Di-ethylglycine and its higher homologs and 1-amino cycloalkane carboxylic acids.
6. A peptide according to claim 4 or 5, wherein leucine is replaced with isoleucine.
7. A peptide according to claim 4, 5 or 6, wherein tryptophan is replaced with D- tryptophan.
8. A peptide according to any one of claims 4 to 7, wherein the peptide is used for the treatment of cancer of colon, breast, ovary, lung, prostate or leukemias.
9. A peptide having the general formula $\text{D-Arg}^1\text{-Pro}^2\text{-Lys}^3\text{-Pro}^4\text{-D-Phe}^5\text{-Gln}^6\text{-D-Trp}^7\text{-Phe}^8\text{-D-Trp}^9\text{-Leu}^{10}\text{-Leu}^{11}\text{-NH}_2$.

10. A peptide according to claim 9, wherein the length of the peptide is 5 to 11 amino acids and amino acids at positions 1 to 11 is replaced by Dxg and/or Aib at any one or more given positions, wherein Dxg represents cyclic and acyclic dialkylated Glycines such as Aib, Di-ethylglycine and its higher homologs and 1-amino cycloalkane carboxylic acids.
11. A peptide according to claim 9 or 10, wherein the peptide is used for the treatment of cancer of colon, breast, ovary, lung, prostate or leukemias.
12. A peptide having the general formula Ala¹-Gly²-Cys³-Lys⁴-Asn⁵-Phe⁶-Phe⁷-D-Trp⁸-Lys⁹-Thr¹⁰-Phe¹¹-Thr¹²-Ser¹³-D-Cys¹⁴ (disulfide 3-14 bridge).
13. A peptide according to claim 12, wherein amino acids at positions 1 to 14 may be replaced by Dxg and/or Aib at any one or more given positions, wherein Dxg represents cyclic and acyclic dialkylated Glycines such as Aib, Di-ethylglycine and its higher homologs and 1-amino cycloalkane carboxylic acids.
14. A peptide according to claim 12 or 13, wherein the peptide is used for the treatment of cancer of colon, breast, ovary, lung, prostate or leukemias.
15. A peptide having the general formula D-Phe¹-Cys²-Tyr³-D-Trp⁴-Orn⁵-Thr⁶-Pen⁷-Thr⁸-NH₂ (Disulfide 2-7 bridge).
16. A peptide according to claim 15, wherein amino acids at positions 1 to 8 is replaced by Dxg and/or Aib at any one or more given positions, wherein Dxg represents cyclic and acyclic dialkylated Glycines such as Di-ethylglycine and its higher homologs and 1-amino cycloalkane carboxylic acids.

17. A peptide according to claim 15 or 16, wherein the peptide is used for the treatment of cancer of colon, breast, ovary, lung, prostate or leukemias.
18. Peptide analogs for the treatment of cancer substantially as hereinbefore described and illustrated with reference to the foregoing Examples.

Dated this 11th day of February 1999.



[K. T. Jose]
OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANTS